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Pesticide Biochemistry and Physiology 89 (2007) 12–19

PESTICIDE Biochemistry & Physiology

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Differential susceptibilities to pyrethroids in field populations of *Chilo suppressalis* (Lepidoptera: Pyralidae)

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Received 26 December 2006; accepted 1 February 2007
Available online 8 February 2007

Abstract

To assess the feasibility of pyrethroids for rice insect control, we examined susceptibilities of six field populations of rice stem borer *Chilo suppressalis* (Walker) to 10 pyrethroids using the topical application method in laboratory in 2004 and 2005. Our results showed that the seven pyrethroids with high fish-toxicity (i.e., β-cyfluthrin, λ-cyhalothrin, β-cypermethrin, deltamethrin, *S*-fenvalerate, α-cypermethrin, and fenpropathrin) were more effective against *C. suppressalis* than the three compounds with low fish-toxicity (i.e., cycloprothrin, etofenprox, and silafluofen). The results also showed that all 10 of the pyrethroids were much more effective than methamidophos and monosultap for *C. suppressalis* control. In addition, we found that susceptibilities of some field populations of *C. suppressalis* to some high fish-toxicity pyrethroids were significantly reduced, and our results indicated that a Ruian (RA) field population showed a year-to-year variation in susceptibility to most tested pyrethroids between 2004 and 2005. Our data indicated that the tolerance levels increased dramatically in RA population, especially to β-cyfluthrin and deltamethrin. This study provided the first assessment of resistance to pyrethroids in field populations of *C. suppressalis*. In addition, a close correlation between resistance ratios to the 10 compounds and differences of the structures of these compounds was established in the RA05 population, which was resistant to most of the pyrethroids tested while it was still very susceptible to fenvalerate with no cross resistance. Finally, the feasibility and precaution were discussed in selecting pyrethroids as alternatives to replace high toxicity organophosphates for *C. suppressalis* control and insecticide resistance management.
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Keywords: *Chilo suppressalis*; Pyrethroids; Toxicity; Susceptibility/resistance; Cross resistance

1. Introduction

The rice stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), is one of the economically important rice insects in China [1]. Currently, control of *C. suppressalis* relies mainly on chemical insecticides, especially organophosphates (OP). Due to their high toxicity risk to non-target organisms and environment, some high toxicity OPs will be banned in 2007 by the Ministry of Agriculture in China. One of which is methamidophos that was used to control *C. suppressalis*. In addition, populations of *C. suppressalis* in many rice production regions of China have developed high levels of resistance to monosultap and triazophos, which are two conventional insecticides for chemical control of *C. suppressalis* [2–16]. Resistance to a highly effective novel insecticide fipronil has also been observed in some field populations in the last 10 years [17–19]. Therefore, it is urgent to find alternatives to replace the high toxicity OPs and other conventional insecticides (monosultap and triazophos), which showed increasing development of the resistance in *C. suppressalis.*

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doi:10.1016/j.pestbp.2007.02.002

In addition to a few low toxicity OPs and novel insecticides, pyrethroids are considered to be potential alternatives for the control of rice insects. Pyrethroids may have many disadvantages when applied in rice fields, such as the toxicity risk to beneficial aquatic organisms and potential causing of planthopper resurgence. As low toxicity pyrethroids are highly desirable, chemical companies currently tend to pursue low fish toxicity products, such as the non-ester pyrethroids. Etofenprox (MTI-500), cycloprothrin, silafluofen, and phenothrin, which have been successfully adopted in rice paddies in Japan and some Southeast Asian countries [20–23]. The susceptibility of important pest populations to insecticides should be investigated before large-scale pesticide implementation [24]. Therefore, in order to provide scientific basis for the assessment of the feasibility of applying pyrethroids for rice insect control, we carried out research to examine 10 pyrethroid insecticides as possible alternatives for replacing highly toxic OPs. This study was also designed to determine the susceptibilities of representative field populations of *C. suppressalis* to selected pyrethroids and to establish a baseline for monitoring and managing resistance development in *C. suppressalis*.

2. Materials and methods

2.1. Insects

In 2004 and 2005, six populations of *C. suppressalis* were collected from rice fields in four sites covering three provinces. LYG04 and LYG05 populations were collected in 2004 and 2005 in Lianyungang, Jiangsu Province. Population CS04 was collected in 2004 from Changshu, Jiangsu Province. RA04 and RA05 populations were collected in 2004 and 2005 in Ruian, Zhejiang Province. GL05 population was collected in 2005 from Guiling, Guangxi Autonomous Region. These populations represented different rice production regions as LYG and CS for eastern China, RA for Southeastern China, and GL for Southern China. All insects were maintained in laboratory using the rice seedlings rear method [25], and the rearing conditions were maintained at 28 ± 1 °C and 16:8 (L:D) h.

2.2. Insecticides

The technical grade insecticides, listed in Table 1, were used for bioassays with *C. suppressalis*. A total of 10 pyrethroids were selected, seven of which are highly toxic to fish and the other three pyrethroids (cycloprothrin, etofenprox, and silafluofen) have low toxicity to fish. In addition, two organophosphates, methamidophos and triazophos, and a nereistoxin analogues, monosultap, were included for comparison of pyrethroids with different insecticide classes.

2.3. Bioassays

The topical application method [26] was used to conduct bioassay on each population of *C. suppressalis*. Middle fourth instar larvae with body weight ranging 6–9 mg per larva were used as a standard larval stage in the bioassays [7]. Larvae were placed into Petri dishes (5 cm) containing a piece ($1 \times 1 \times 0.3$ cm) of artificial diet. The components of the artificial diet reported by Tan [27] were revised from the recipe reported by FAO [26]. Insecticides were diluted into a series of concentrations with acetone, except monosultap with a mixture of acetone and water at ratio of 1:1 because of its low solubility in acetone. A droplet of 0.04 μl insecticide solution was applied topically on the dorsal part of larval middle abdomen with a capillary microapplicator [26]. Three replicates were used and in each replication 10 larvae were treated for each insecticide concentration. Control insects were treated with acetone alone or with a mixture of acetone and water as control for the treatments of monosultap. The rearing conditions for treated larvae were controlled at 28 ± 1 °C and 16:8 (L:D) h. Mortality was recorded 96 h after treatment for monosultap and 48 h after treatment for other insecticides. Larvae

Table 1
The details of the insecticides tested

Insecticides	Chemical group	Technical grade (AI) (%)	Companies
α-Cypermethrin	Cyclopropane carboxylate pyrethroids with ethenyl	96.48	Jiangsu Yangnong Chemical Co., Ltd.
β-Cyfluthrin	Cyclopropane carboxylate pyrethroids with ethenyl	92	Jiangsu Yangnong Chemical Co., Ltd.
β-Cypermethrin	Cyclopropane carboxylate pyrethroids with ethenyl	93.2	Jiangsu Yangnong Chemical Co., Ltd.
Cycloprothrin	Cyclopropane carboxylate pyrethroids without ethenyl	89	Jiangsu Yangnong Chemical Co., Ltd.
Deltamethrin	Cyclopropane carboxylate pyrethroids with ethenyl	98	Jiangsu Yangnong Chemical Co., Ltd.
Etofenprox	Non-ester pyrethroids	90	Jiangsu Yangnong Chemical Co., Ltd.
Fenpropathrin	Cyclopropane carboxylate pyrethroids without ethenyl	94.8	Nanjing Redsun Co., Ltd.
λ-Cyhalothrin	Cyclopropane carboxylate pyrethroids with ethenyl	96.4	Jiangsu Yangnong Chemical Co., Ltd.
Methamidophos[a]	Organophosphates	73	Shandong Huayang Technology Co., Ltd.
Monosultap[a]	Nereistoxin analogues	90	Hunan Jinyuan Pesticide Chemical Plant
S-fenvalerate	Isovalerate pyrethroids	95	Jiangsu Institute of Ecomones Co., Ltd.
Silafluofen	Non-ester pyrethroids	96.1	Jiangsu Yangnong Chemical Co., Ltd.
Triazophos[a]	Organophosphates	80.5	Zhejiang Yongnong Chemical Industry Co., Ltd.

[a] Insecticides were tested as references.

were counted as dead if no response was observed after probing with a pin.

2.4. Statistical analysis

The PoloPlus software [28] was used for probit analysis of dose–response data. The LYG04 population was used as susceptible population, because it was very susceptible to most insecticides tested, such as triazophos, chlorpyrifos, fipronil, and other insecticides (RR < 3-fold, unpublished data). The resistance ratio (RR) was calculated by dividing the LD_{50} of a field population by the corresponding LD_{50} of the susceptible strain (LYG04). Resistance levels were classified based on Shen's standard [29] as: susceptible: RR < 3-fold; minor resistance: RR = 3–5-fold; low resistance level: RR = 5–10-fold; medium resistance level: RR = 10–40-fold; high resistance level: RR = 40–160-fold; extremely high resistance level: RR > 160-fold. Data were further statistically analyzed with SAS program [30]. Proc Mixed and Proc GLM procedures were used for variance analyses. Mean separation was conducted using SAS Proc Means/LSD or Lsmeans separation programs at $P < 0.05$.

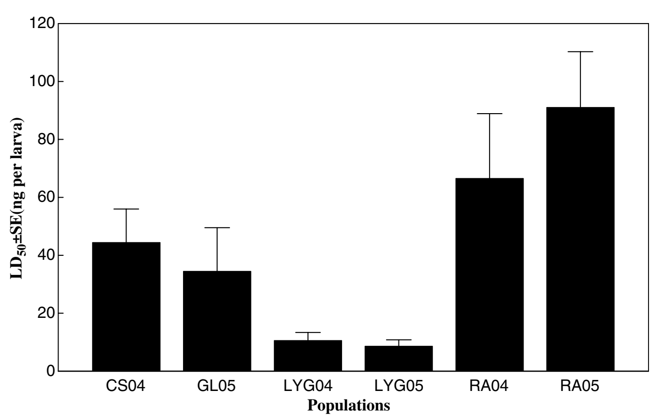

Fig. 1. Differential susceptibility (pooled LD_{50} from 10 pyrethroid insecticides) of six field populations of *Chilo suppressalis*.

3. Results

3.1. Susceptibility variation of different field populations

Populations collected from different regions exhibited significantly different responses to selected pyrethroid insecticides ($F = 5.21$, df = 5, $P < 0.001$). LYG04 ($LD_{50} = 10.57$ ng/larva) and LYG05 ($LD_{50} = 8.62$ ng/larva) populations were relatively susceptible (Fig. 1), while the RA04 ($LD_{50} = 66.53$ ng/larva) and RA05 ($LD_{50} = 91.04$ ng/larva) populations were more tolerant to the insecticides. Susceptibility levels of CS04 ($LD_{50} = 44.42$ ng/larva) and GL05 ($LD_{50} = 34.47$ ng/larva) were located between LYG and RA populations (Fig. 1).

3.1.1. LYG populations

Two LYG populations collected in 2004 (LYG04) and in 2005 (LYG05) had similar susceptibilities to individual pyrethroid (Table 2). Among the 10 pyrethroids and three other insecticides tested, β-cyfluthrin was the most effective insecticide against *C. suppressalis* ($LD_{50} = 0.09$ ng/larva and 1.6 ng/larva, respectively, in LYG04 and LYG05 populations), and followed by λ-cyhalothrin, β-cypermethrin, deltamethrin, and *S*-fenvalerate ($LD_{50} = 0.68$–0.75 ng/larva). α-Cypermethrin and fenpropathrin showed less effectiveness with LD_{50} ranging from 1.7 to 7.4 ng/larva. Cycloprothrin, etofenprox, and silafluofen exhibited the least efficacy against *C. suppressalis* ($LD_{50} = 15.23$–42.23 ng/larva). These three pyrethroids were also less effective than an organophosphate triazophos ($LD_{50} = 6.1$–6.9 ng/larva), but still more effective than methamidophos ($LD_{50} = 100$ ng/larva) and monosultap ($LD_{50} = 1270$ ng/larva).

Based on LD_{50}s to all 10 pyrethroids, LYG populations showed no significant year to year difference ($F = 0.28$, df = 1, $P > 0.05$ [0.5957]). Considering that the LYG population was very susceptible to most pyrethroids tested and other insecticides, such as triazophos, chlorpyrifos, fipronil, etc. (RR < 3-fold, unpublished data), the LYG04 population was used as susceptible population and as a

Table 2
Dose-responses ($LD_{50} \pm SE$)[A] of field populations of *C. suppressalis* to 10 selected pyrethroids

Insecticides	LYG04	LYG05	RA04	RA05	CS04	GL05
α-Cypermethrin	1.7 ± 0.25 c	3.73 ± 0.83 d	11.53 ± 1.25 c	23.93 ± 6.57 d	15.30 ± 2.10 de	—
β-Cyfluthrin	0.09 ± 0.01 c	0.16 ± 0.02 d	1.63 ± 0.30 c	13.10 ± 0.82 d	0.58 ± 0.06 e	2.30 ± 0.35 b
β-Cypermethrin	0.75 ± 0.04 c	1.29 ± 0.30 d	11.97 ± 0.79 c	31.17 ± 3.43 d	10.17 ± 1.33 de	—
Cycloprothrin	24.50 ± 3.49 b	15.23 ± 2.86 bc	59.17 ± 3.66 c	179.00 ± 21.30 b	67.43 ± 5.98 c	93.53 ± 9.57 a
Deltamethrin	0.69 ± 0.02 c	1.40 ± 0.12 d	7.00 ± 0.81 c	84.77 ± 20.85 c	8.30 ± 0.62 e	7.57 ± 0.17 b
Etofenprox	42.43 ± 1.21 a	19.57 ± 2.64 b	185.00 ± 33.02 b	193.83 ± 42.53 b	99.13 ± 10.05 b	—
Fenpropathrin	4.70 ± 0.64 c	7.40 ± 1.10 cd	6.90 ± 1.25 c	37.50 ± 1.71 cd	28.70 ± 4.61 d	—
λ-Cyhalothrin	0.68 ± 0.09 c	0.75 ± 0.22 d	3.87 ± 0.50 c	22.77 ± 1.68 d	2.23 ± 0.15 e	—
S-fenvalerate	1.11 ± 0.14 c	0.80 ± 0.12 d	4.37 ± 0.62 c	2.47 ± 0.52 d	7.80 ± 0.91 e	—
Silafluofen	28.53 ± 7.39 b	35.90 ± 7.50 a	373.83 ± 68.13 a	321.83 ± 19.62 a	204.53 ± 16.56 a	—

— Experiments were not conducted due to limited insect collections.
[A]Means followed by same letters are not significantly different at $P = 0.05$ within column.

baseline for comparing relative tolerance levels in other field populations.

3.1.2. RA populations

RA04 population was most sensitive to β-cyfluthrin ($LD_{50} = 1.63$ ng/larva; Table 2). The population showed similar dose response to seven other pyrethroids ($LD_{50} = 3.87$–59.17 ng/larva for λ-cyhalothrin, *S*-fenvalerate, fenpropathrin, deltamethrin, α-cypermethrin, β-cypermethrin, and cycloprothrin). Etofenprox showed significantly lower toxicity ($LD_{50} = 185$ ng/larva) than above pyrethroids, and higher toxicity than silafluofen ($LD_{50} = 373.83$ ng/larva), against *C. suppressalis* ($F = 25.82$, df = 9, $P < 0.0001$). The reference insecticide triazophos ($LD_{50} = 240$–460 ng/larva) had similar effectiveness as silafluofen. However, silafluofen was far more effective than methamidophos ($LD_{50} = 800$ ng/larva) and monosultap ($LD_{50} = 15946$ ng/larva).

The sensitivities of RA05 population to 10 pyrethroids (Table 2) were as: *S*-fenvalerate, β-cyfluthrin, λ-cyhalothrin, α-cypermethrin, β-cypermethrin, fenpropathrin ($LD_{50} = 2.47$–37.5 ng/larva) > deltamethrin ($LD_{50} = 84.77$ ng/larva) > cycloprothrin and etofenprox ($LD_{50} = 179$–193.83 ng/larva) > silafluofen ($LD_{50} = 321.83$ ng/larva) ($F = 35.69$, df = 9, $P < 0.0001$).

3.1.3. CS04 population

CS04 population was most sensitive to β-cyfluthrin (Table 2; $LD_{50} = 0.58$ ng/larva) and it had similar sensitivities to λ-cyhalothrin, *S*-fenvalerate, deltamethrin, β-cypermethrin, and α-cypermethrin ($LD_{50} = 2.23$–15.3 ng/larva). Significantly lower sensitivities ($F = 95.43$, df = 9, $P < 0.0001$) were detected in the insect for fenpropathrin, cycloprothrin, etofenprox, and silafluofen ($LD_{50} = 28.7$–204.53 ng/larva). Comparison with the reference insecticides indicated that most pyrethroids were more effective than triazophos ($LD_{50} = 46$ ng/larva) and monosultap ($LD_{50} = 2409$ ng/larva) against *C. suppressalis*.

3.1.4. GL05 population

Because a small number of insects of GL05 population were collected in 2005, only three pyrethroids were used for bioassays. The ranking of toxicities (Table 2) is as below: β-cyfluthrin > deltamethrin > cycloprothrin ($LD_{50} = 2.3$, 7.57, and 93.53 ng/larva, respectively). These pyrethroids were more effective against *C. suppressalis* than triazophos ($LD_{50} = 270$ ng/larva).

3.2. Efficacy variation of different pyrethroids

Ten selected pyrethroids exhibited significant variations of toxicological effects (Fig. 2) against *C. suppressalis* ($F = 17.46$, df = 9, $P < 0.0001$). Based on general dose responses of six populations, β-cyfluthrin was the most effective pyrethroid ($LD_{50} = 2.98$ ng/larva).

S-fenvalerate, λ-cyhalothrin, β-cypermethrin, α-cypermethrin, fenpropathrin, and deltamethrin had similar

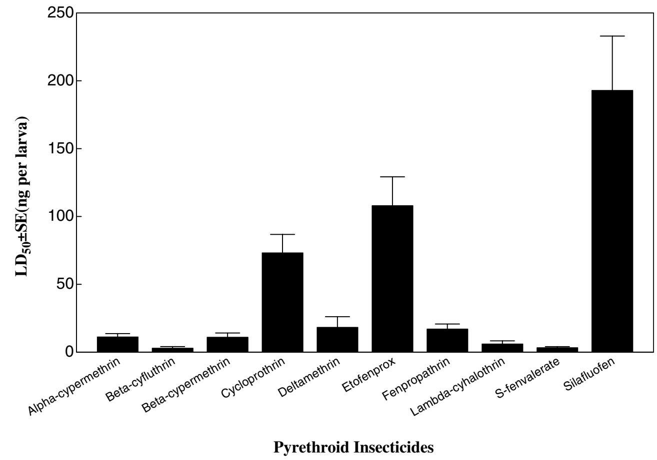

Fig. 2. Toxicological effects (LD50) of 10 pyrethroid insecticides against *C. suppressalis* (pooled LD_{50} from six populations).

toxicity against *C. suppressalis* ($LD_{50} = 3.31$–18.29 ng/larva). Cycloprothrin and etofenprox showed less effectiveness with $LD_{50} = 73.14$ and 107.99 ng/larva, respectively. Silafluofen was the least effective insecticide ($LD_{50} = 192.93$ ng/larva) among 10 tested pyrethroids.

Chemical structures had significant influence on pyrethroid toxicity against *C. suppressalis* ($F = 39.83$, df = 3, $P < 0.0001$). Among the 10 pyrethroids, β-cyfluthrin, deltamethrin, β-cypermethrin, λ-cyhalothrin, and α-cypermethrin have similar chemical structures including four functional groups (α-cyano, ethenyl, cyclopropane carboxylate, and phenoxybenzyl). This group of pyrethroids showed higher toxicity ($LD_{50} = 9.98$ ng/larva, Fig. 2). The fenvalerate has modification in the acid part and it also exhibited higher toxicity against *C. suppressalis* with relative low LD_{50} values (3.31 ng/larva). Two pyrethroids, fenpropathrin, and cycloprothrin, do not have the ethenyl group and their LD_{50} values were approximately 47.64 ng/larva. Silafluofen and etofenprox are non-ester pyrethroids, and their toxicity to the insect was relatively low ($LD_{50} = 150.46$ ng/larva).

3.3. Variations of resistance ratios among populations

Resistance ratios (RR) were calculated for comparisons of population susceptibilities to 10 selected pyrethroids (Table 2). Because LYG04 was relatively susceptible and sensitive to 10 pyrethroids, its LD_{50} values were used as references for calculation of RRs of the other populations. Variance analysis indicated that the six populations had significantly different RRs ($F = 11.26$, df = 5, $P < 0.0001$). The RA05 population had the highest RR (40.39-fold, Fig. 3), which was significantly different from the RRs of all other five populations ($P < 0.0001$). The RRs for RA05 population ranged from 2.23-fold to *S*-fenvalerate to 155.15-fold to β-cyfluthrin. LYG05 still maintained susceptibility and the RR only increased 1.4-fold (0.47–2.33). CS04, RA04, and GL05 populations increased RRs by 7.23

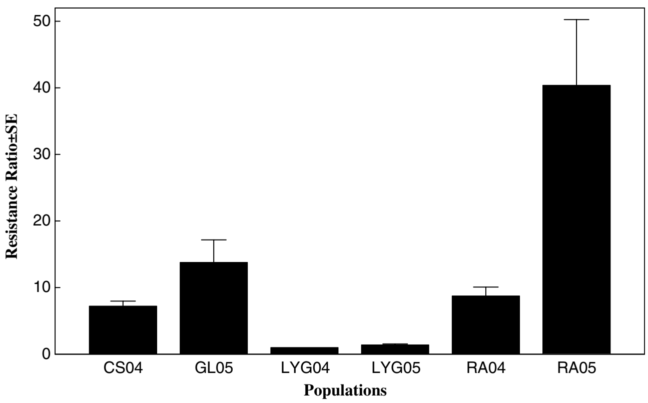

Fig. 3. Differential resistance ratios (pooled from 10 pyrethroid insecticides) of six field populations of *C. suppressalis*.

(2.33–13.51), 8.75 (1.48–18.69), and 13.80 (4.01–26.48)-fold, respectively (Table 3 and Fig. 3). Year-to-year variation analysis indicated that RA05 population significantly ($F = 10.10$, df = 1, $P < 0.01$) increased RR by 4.61-fold compared with RA04 population, which had already showed 8.75-fold increase of RR from the level of LYG04 population.

3.4. Variations of resistance ratios among pyrethroids

Resistance ratios (RR) were also calculated for comparisons of resistance ratios of 10 selected pyrethroids in the six field populations (Table 3). Ten pyrethroid insecticides exhibited significant variation for RR development ($F = 3.11$, df = 9, $P < 0.01$). Among the 10 pyrethroids (Fig. 4), β-cyfluthrin and deltamethrin had the highest rate of RR increases (35.01- and 26.16-fold, respectively) in all six (pooled) populations. They reached 155.15- and 120.87-fold in the RA05 population. *Chilo suppressalis* had very high rate of RR to β-cypermethrin (14.80-fold), and minor to low levels of RR increases to other seven pyrethroids, ranging from 2.55-fold to etofenprox to 9.41-fold to λ-cyhalothrin (Fig. 4). *Chilo suppressalis* also had significantly different RRs for different pyrethroid groups ($F = 4.39$, df = 3, $P < 0.01$). *Chilo suppressalis*

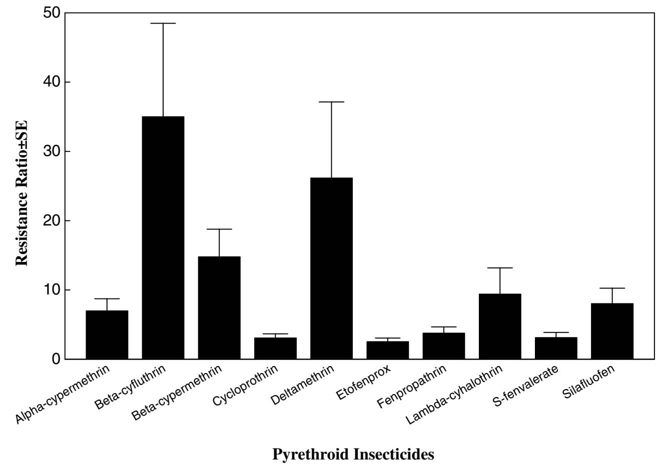

Fig. 4. Resistance ratios of *C. suppressalis* to 10 pyrethroid insecticides (pooled from six populations).

showed the highest RRs to the group of β-cyfluthrin, deltamethrin, β-cypermethrin, λ-cyhalothrin, and α-cypermethrin (RR = 19.37). Other three groups had minor to low levels of RR increases, ranging from 3.40- to 5.30-fold.

4. Discussion

In this study, variable susceptibilities were detected in different populations to different pyrethroid insecticides. Among the six populations examined, LYG populations were relatively susceptible, and they maintained low susceptibility to most pyrethroids over a two-year period (2004–2005). Unlike the LYG populations, the RA populations were particularly prone to change their susceptibility to many pyrethroids while they were still able to maintain susceptibility to certain pyrethroids, such as *S*-fenvalerate and etofenprox. The RA04 population exhibited greater variation in response to 10 selected pyrethroids. The tolerance level to β-cyfluthrin increased to 155-fold after one year. One of the potential causes was the intensity of pesticide applications. In the region where the RA populations were collected for this study, the rice stem borer produces four generations in a year. Pesticides are applied more than

Table 3
Resistance ratios[A] of field populations of *C. suppressalis* to 10 selected pyrethroids

Insecticides	LYG04	LYG05	RA04	RA05	CS04	GL05
α-Cypermethrin	1	2.33 ± 0.67 a	6.92 ± 0.79 cd	15.46 ± 5.71 cde	9.26 ± 1.66 bc	—
β-Cyfluthrin	1	1.96 ± 0.38 ab	18.69 ± 1.76 a	155.15 ± 18.21 a	6.78 ± 0.82 cd	26.48 ± 1.80 a
β-Cypermethrin	1	1.77 ± 0.50 ab	16.20 ± 1.82 ab	41.50 ± 2.96 c	13.51 ± 1.26 a	—
Cycloprothrin	1	0.64 ± 0.13 cd	2.50 ± 0.34 cd	7.48 ± 1.07 de	2.88 ± 0.50 ef	4.01 ± 0.73 c
Deltamethrin	1	2.03 ± 0.21 ab	10.16 ± 1.36 bc	120.87 ± 26.59 b	12.01 ± 1.09 ab	10.92 ± 0.07 b
Etofenprox	1	0.47 ± 0.07 d	4.41 ± 33.02 b	4.55 ± 0.96 de	2.33 ± 0.19 f	—
Fenpropathrin	1	1.59 ± 0.19 abc	1.48 ± 0.21 d	8.22 ± 0.91 de	6.61 ± 2.03 cde	—
λ-Cyhalothrin	1	1.08 ± 0.24 bcd	6.00 ± 1.34 cd	35.47 ± 7.86 cd	3.47 ± 0.76 def	—
S-fenvalerate	1	0.76 ± 0.17 cd	4.17 ± 0.93 cd	2.37 ± 0.70 e	7.37 ± 1.44 c	—
Silafluofen	1	1.35 ± 0.26 abcd	16.99 ± 7.79 ab	12.78 ± 3.00 cde	8.07 ± 1.74 c	—

— Experiments were not conducted due to limited insect collections.
[A] Means followed by same letters are not significantly different at $P = 0.05$ within column.

five times a year, which is the highest level of pesticide applications in China for the control of this pest. However, the correlation between intensity of pesticide applications and resistance development has not been established. Future study is also needed to establish resistant colonies to investigate whether and how the tolerance or resistance is inherited and to examine molecular and genetic mechanisms if the resistance is confirmed.

Pyrethroids, except for etofenprox, have been banned for use in rice paddies for a long time in China [22]. However, because of the emergency outbreak of a few rice insect pests, such as rice leaf roller *Cnaphalocrocis medinalis* Guenee and brown planthopper *Nilapavata lugens* during the past a few years, some pyrethroids like λ-cyhalothrin and β-cypermethrin were privately used by farmers for the control of these rice pests. Geographic variation and sensitivity of *C. suppressalis* to pyrethroids have never been investigated before. In this study, we collected *C. suppressalis* in four representative rice paddies. Each location had a different application level for the chemical control. Responses to 10 selected pyrethroids were examined by measuring LD_{50} values. Shift of the sensitivities was further surveyed after a one year period in two locations, LYG and RA. The results from this study indicated that *C. suppressalis* had variable sensitivities to the 10 selected pyrethroids. This study also brought public attention to the fact that resistance in *C. suppressalis*, particularly in the RA population, is occurring at an alarming pace. Fast resistance development in *C. suppressalis* might be a result from misuse and unauthorized applications of pyrethroids in rice paddies by farmers. Our results showed that susceptibilities of some field populations of *C. suppressalis* (i.e., RA and CS populations) to some pyrethroids (i.e., β-cyfluthrin, deltamethrin, λ-cyhalothrin, β-cypermethrin, α-cypermethrin, *S*-fenvalerate, and fenpropathrin) were significantly reduced by a certain range. More than 100-fold (RR = 155.15-fold) reduction in sensitivity to β-cyfluthrin was observed in RA05 population. Year-to-year comparison of the sensitivities of *C. suppressalis* from the same location (RA04 vs. RA05) provided evidence that evolution of pyrethroid resistance in *C. suppressalis* reached a surprising speed.

However, no matter what resistance level this insect had developed too many pyrethroids, *C. suppressalis*, including RA population, was still very susceptible to fenvalerate. This phenomenon indicated that no cross-resistance to fenvalerate was developed in *C. suppressalis*, though the RA population has quickly and substantially reduced sensitivities to β-cyfluthrin, deltamethrin, and other pyrethroids. The detail structures of the 10 pyrethroids were further analyzed and compared with the corresponding resistance ratios. To our surprise, we found a close correlation between the resistance ratios to 10 compounds in the RA05 population and the variations of the structures of these compounds. The five compounds (β-cyfluthrin, deltamethrin, β-cypermethrin, λ-cyhalothrin, and α-cypermethrin) have similar chemical structures including four functional groups (α-cyano, ethenyl, cyclopropane carboxylate, and phenoxybenzyl) and their RR values ranged from 15.46- to 155.15-fold. Fenpropathrin and cycloprothrin do not have the ethenyl group and their RR values were between 7.48- and 8.22-fold. Also, the RA05 population showed low level of resistance to silafluofen and etofenprox (RR = 12.78- and 4.55-fold), which are non-ester pyrethroids without α-cyano. The acid part of fenvalerate is not cyclopropane carboxylate but isovalerate and the RR value of the population to fenvalerate was 2.37-fold, indicating the population was very susceptible to this compound. This analysis shows that the cross-resistance between pyrethroids in *C. supressalis* is closely related with the similarity of their chemical structures. However, the correlation is not significant in the population CS04 with medium level of pyrethroid resistance. This might be the result of different application levels of pyrethroids. Zhang and Han [31] also reported that the deltamethrin-resistant *Musca domestica vicina* L. strain not only conferred serious cross resistance to α-cypermethrin and cypermethrin, but also developed a low level of cross resistance to fenvalerate. Our suggestion might not totally agree with many others [32–35] showing the existence of significant cross resistance between fenvalerate and other pyrethroids, such as deltamethrin, β-cypermethrin, λ-cyhalothrin, etc. In many cases, correlation might lead to a false judgment on existence of cross-resistance. Shen and Wu [29] suggested that it is practical to rely more on insecticide application history in a field when cross-resistance needs to be determined. In this study, we found that the resistance in *C. suppressalis* seemed to be correlated between triazophos and pyrethroids. However, these two insecticides were often rotated. It would be premature to conclude that a cross-resistance exists between these compounds if the conclusion is drawn based on correlation instead of examination of application history of these two chemicals. In addition, it is very likely that different insect species may have different mechanisms for developing resistance and cross-resistance, which might account for the disagreement between our result and other observations. In spite of this, further study is still necessary to confirm whether the cross-resistance between fenvalerate and other pyrethroids does not exist in *C. suppressalis*.

Evolution of pyrethroid resistance in insects has great potential to nullify chemical control. It was well documented that insects are able to develop resistance rapidly [36] and that resistance can reach a higher level than resistance to other insecticides [37]. Synthetic pyrethroids are very potent. Even a small dose may be toxic enough to achieve substantial control of many important pests [38]. But, reduced efficacy as the consequence of the resistance development would prompt increasing of application amount and spray frequency. Subsequently, more toxic chemicals are released into the environment. As vulnerable aquatic organisms and natural enemies are adversely affected by the insecticides, population resurgence and secondary pest outbreak can readily happen. The situation was noticeable especially in the last two years when serious outbreak of brown planthopper, *N. lugens* happened in

China. Misuse of the pyrethroids might be the one of the potential reasons for the outbreak [39].

In this study, we found that some field populations of *C. suppressalis* were already resistant to most pyrethroids which have high toxicity to fishes and other aquatic organisms. If these pyrethroids continue to be used to control the resistant population, it could lead to more serious consequence, such as above mentioned population resurgence and secondary pest outbreak. Another serious problem is the development of multiple resistances in some field populations of *C. suppressalis*, especially in the Southeast Zhejiang rice area, from where the RA population was collected. According to our other study (unpublished data), RA05 population has already developed a high level resistance to conventional insecticides, such as monosultap, triazophos, fenitrothion, and endosulfan (40.7–74.9-fold). The RA05 population also developed medium level resistance to fipronil, chlorpyrifos, and other OPs (10.0–27.7-fold). In this study, we found that the RA population had developed various resistant levels (from low to high level) to several pyrethroids. To deal with a population with multiple resistances, the Integrated Resistance Management (IRM) must be implemented, including the strategy to limit or avoid the use of insecticides, to which the target pest has already developed multiple resistances. However, because of their high efficacy against *C. suppressalis*, pyrethroids may still be considered as available alternatives for temporarily replacing high toxicity OPs and other conventional insecticides, but it is necessary to develop some better techniques and strategies to resolve the risk problems caused by the application of pyrethroids in rice fields.

In spite of toxicity and resistance problems for many pyrethroids, a few compounds, such as cycloprothrin, etofenprox, and silafluofen have a relatively low toxic effect on fishes, and they should not be excluded for further evaluation for their potential use in rice field. Etofenprox has been registered in China since 2004 for the control of planthoppers and weevils in rice fields [40]. Laboratory bioassays and field trials showed that etofenprox was effective against many insect pests on rice [41–44]. Our data also showed that these low fish-toxicity pyrethroids had higher efficacy against *C. suppressalis* than monosultap and methamidophos. They also showed similar or better effect (Tables 2 and 3) against triazophos-resistant populations (RA04 and RA05 populations) than triazophos. Besides these, all tested populations were susceptible to the three low fish-toxicity pyrethroids, and the majority of the RR values were lower than 5-fold, i.e., no more than minor resistance level.

In summary, we examined the biological effects of 10 pyrethroid insecticides to six populations of *C. suppressalis* collected in four representative locations. By analyzing chemical structures of the pyrethroids, we established correlation between structure and cross resistance, which is an important component for the implementation of candidate insecticide in rice fields. Three low fish-toxicity pyrethroids, cycloprothrin, etofenprox, and silafluofen, are potential candidates for replacing high toxicity organophosphates because of their low resistance ratios and better efficacy against the insect as well. Our results also indicated that precautions should be taken to avoid potential chemical control failure due to rapid resistance development in the target populations. More studies should be conducted to minimize the risk of environment toxicity, resistance development, and outbreak of other rice insects.

Acknowledgments

We thank Ruian Plant Protection station of Zhejiang, Lianyungang station and Changshu station of Jiangsu, and Quanzhou station of Guiling, Guangxi, for helping collect test populations of *C. suppressalis*. The authors are grateful to Dr. Fangneng Huang of Louisiana State University and Dr. Xinzhi Ni of USDA-ARS Tifton GA for their comments and suggestions for improving early version of this manuscript. This research was funded by the Assessment Program of the Feasibility of Pyrethroids to Apply in Rice Paddies, Institute for the Control of Agrochemicals, Ministry of Agriculture of China. Mention of a trademark, warranty, proprietary product or vendor does not constitute a recommendation or endorsement by the USDA and does not imply approval or recommendation of the product to the exclusion of others that may be suitable.

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